

CR-115706

FINAL REPORT

WHITE BLOOD CELL COUNTING SYSTEM.

Contract No. NAS 9-12371

July 15, 1972

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## 1.0 INTRODUCTION

This report summarizes the work conducted to date in the design, fabrication, and test of a prototype white blood cell counting system under contract NAS 9-12371.

## 2.0 SUMMARY

Beckman Instruments, Inc. has designed, fabricated and conducted preliminary tests on a prototype White Blood Cell Counting System. The object of this effort was to develop a system suitable for use in the Skylab IMSS.

The White Blood Cell Counting System consists of three major subsystems. These are:

1. Sample Collection Subsystem
2. Sample Dilution and Fluid Containment Subsystem
3. Cell Counter.

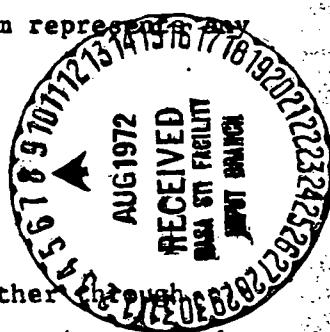
The sample collection, diluting and counting subsystems have been shown to be quite functional and to fulfill the design goals of the program.

The fluid containment subsystem, i.e., sample handling bags, have produced some anomalous results which have been attributed to: (1) adsorption of cells to the walls of the container and (2) inadequate cleaning of the plastic bag material prior to bag fabrication. It is not felt that this bag problem represents any major technical hurdle.

## 3.0 WHITE CELL COUNTING SYSTEM

### 3.1 Sample Collection and Handling

It has been assumed that a blood specimen will be available either through venipuncture or from a capillary puncture. A portion of this specimen will be



measured in a 13  $\mu$ l capillary pipette (Unopette\*). The exterior surfaces of the pipette will be wiped with a disposable tissue to remove any residual blood and then placed into the pipette adapter.

### 3.1.1 Pipette Adapter

The pipette adapter serves two purposes. First, it permits the transfer of the 13  $\mu$ l specimen from the capillary pipette to the specimen container; and second, the transfer of the diluting fluid from the diluter assembly to the container. The adapter illustrated in Figure 3-1 accomplishes the first purpose by permitting the second, i.e., the pipette is placed in the adapter and the adapter attached to the outlet of the diluter. The proper volume of diluent is flushed through the pipette, quantitatively transferring the blood specimen to the sample container. The dilution ratio is 1:430.

### 3.1.2 Sample Container

The sample container design is illustrated in Figure 3-2. As may be seen, the container is a double compartmented bag with the compartments separated by a relatively narrow channel. At the forward end of the bag is found a septum contained in a septum assembly and attached to the container with heat shrink tubing. At the other end of the container is a grommet to which a cord is attached. At the center of the bag is located a clip which serves to occlude the channel. The bag is pre-loaded with 100  $\mu$ l of Zaponin.\*\* In operation, the neck of the bag is inserted into the pipette adapter and firmly pushed until it seats. This assures that the side hole needle of the pipette adapter has penetrated the septum.

### 3.1.3 Diluter

The diluter subsystem permits the dilution of the specimen with the proper volume of an isotonic fluid.\*\* It is essentially a fixed volume syringe with a spring

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\*Becton Dickinson Company, Rutherford, New Jersey.

\*\*Coulter Electronics, Hialeah, Florida.

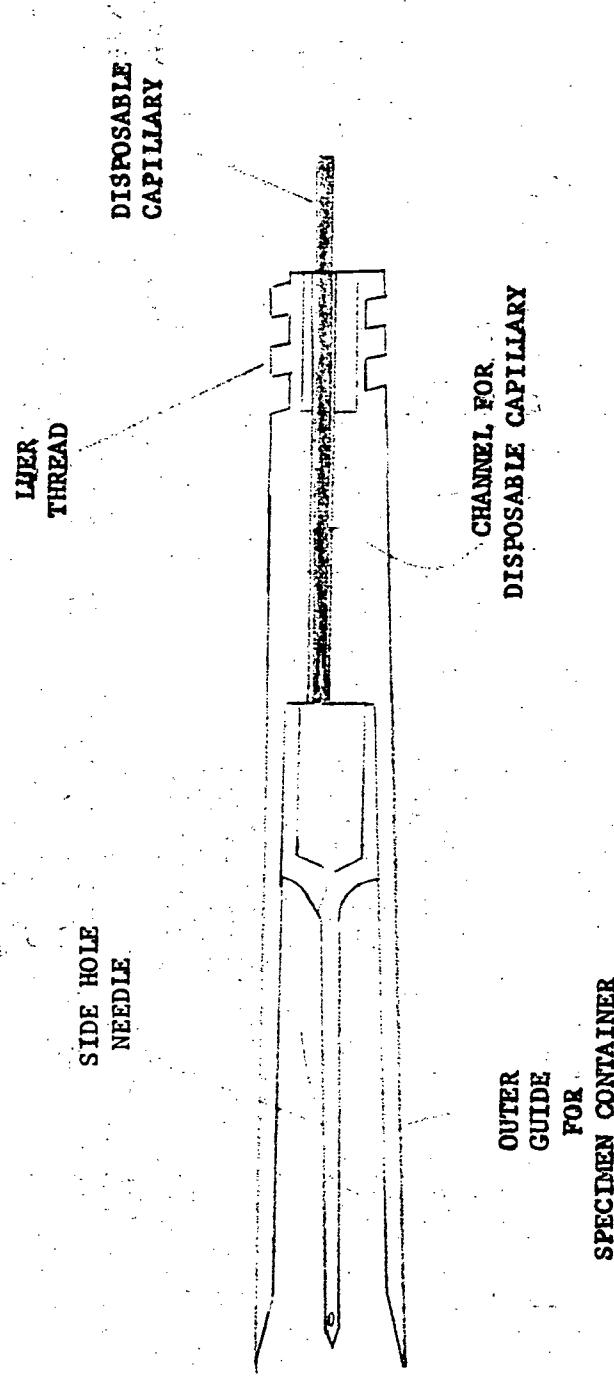


Figure 3-1. Pipette Adapter

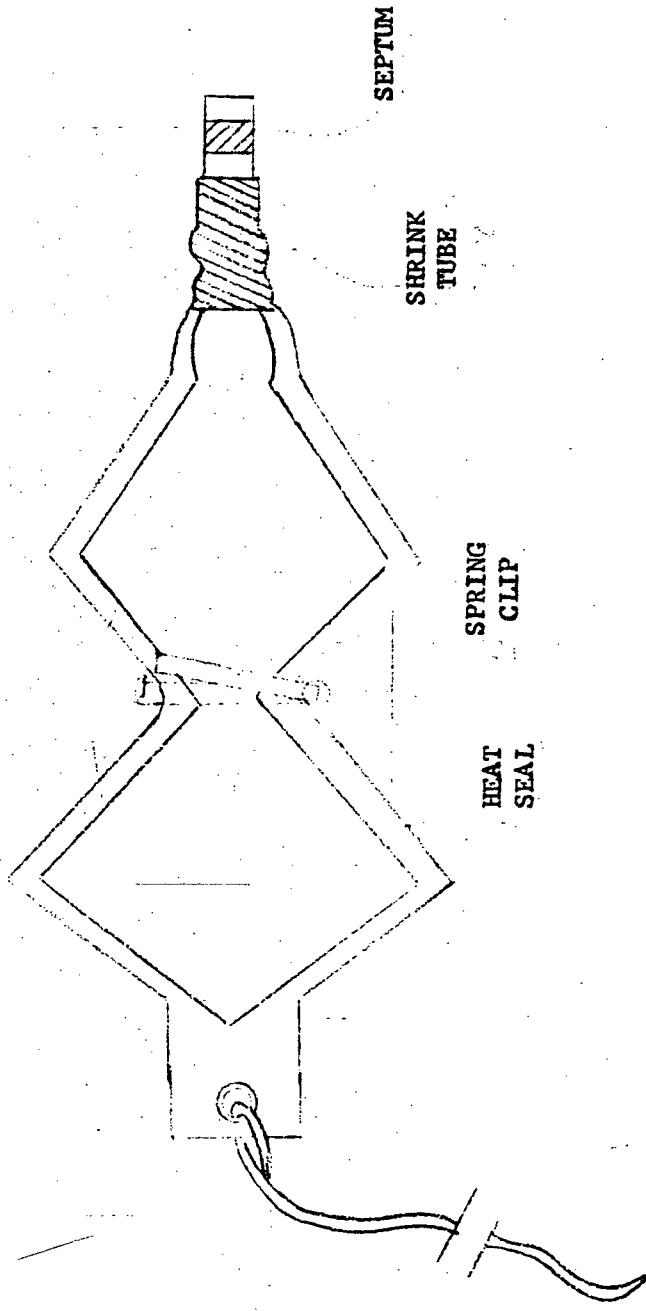


Figure 3-2. Sample Container

loaded piston. The piston is withdrawn against the action of the spring by rotating the handle protruding through the front panel through 180° up the ramp. This permits the dispenser to fill with the diluent fluid from an internal reservoir, which in the flight units will hold adequate fluid for approximately fifteen counts. After a short period of time (5 sec) the handle is rotated in the opposite direction, back down the ramp. The spring load on the dispenser plunger expresses the diluent through the pipette in the pipette adapter and into the sample container through the needle. Since the only passage through the pipette adapter is through the pipette, the specimen is flushed into the sample container ahead of the diluent and the pipette is completely washed free of residual specimen.

### 3.1.4 Specimen

Following the washing of the specimen into the sample container by the diluent, the diluent and specimen are mixed by alternately kneading the upper and lower compartments of the container. This action forces the fluids through the narrow center channel at a relatively high velocity, ensuring that the specimen is well distributed throughout the diluent and that uniform mixing of the specimen and stromatolysin occurs.

Because of the proteinaceous nature of the specimen and because of the stromatolysin, the diluted, lysed sample shows a strong tendency to foam during the mixing procedure. Inasmuch as the bubbles could cause significant interference with the subsequent cell counts, it is necessary to displace the air bubbles to a location in the bag where they will not become a significant problem. This is accomplished by swinging the bag through a circle with a radius of approximately 18 inches for approximately 10-15 seconds. This action moves any bubbles located in the distal compartment to the proximal compartment. After gently slowing the rate of swinging to a gradual stop, the clamp attached to the bag is fastened, occluding the channel and preventing any air from returning to the distal compartment. It is from this compartment that the specimen is aspirated for counting.

### 3.2 Cell Counter

#### 3.2.1 Mechanical Design

The key to the cell counter is its ability to detect and count an event corresponding to the recognition of a white blood cell. In the prototype cell counting system the event is an impedance pulse that occurs as the blood cell, suspended in a saline milieu passes through a narrow orifice between two electrodes. Since the cell is more resistant than the saline, an impedance proportional to the size of the cell pulse results from each passage event. These events are accumulated for a measured volume of fluid and represent the number of cells in that volume. When the proper dilution ratio is employed and the proper volume of diluted fluid is counted, the number of cells counted may be considered to be the count/mm<sup>3</sup> of whole blood.

##### 3.2.1.1 Orifice Assembly

The orifice assembly is the heart of the cell counting system for it is here that the impedance pulse is generated. The neck of the specimen container is firmly inserted into the orifice port, permitting the needle to penetrate the septum. The dilute specimen is then aspirated from the container through an 80  $\mu$  orifice in a laser drilled sapphire, mounted in a plastic housing. Thus, the orifice is an integral part of the fluid flow channel. Also a part of the flow channel and mounted in the plastic housing are two electrodes located on either side of the orifice. Current between these electrodes is altered as a cell traverses the orifice, causing the impedance pulse.

The entire orifice assembly is designed to be a disposable, easily replaceable unit so that in the event of plugging during a mission, the entire unit may be discarded rather than cleaned. For use on earth, however, the orifice is easily accessible and can be cleaned readily.

##### 3.2.1.2 Sample Aspiration

The aspiration of fluid for cell counts presents some unique problems. The aspiration rate must be uniform and not too rapid, and the volume of specimen aspirated (and counted) must be precisely determined. A number of approaches

have been attempted to solve these problems, none of which are particularly appropriate for use in a zero-gravity environment, for, in addition to the requirements for uniform flow rate and extremely accurate volume measurements, we must be concerned about bubble movements, foaming, and sample disposal following the count. The system developed uniquely satisfies all of these requirements.

All systems employ a vacuum to aspirate fluid through the orifice. The most commonly used systems obtain this vacuum through the displacement of a column of mercury. In addition, the mercury falling through a manometer actuates on and off switches to limit the volume of sample counted. This system functions well for its intended application but has several noticeable features that make it entirely inappropriate for use in zero gravity. These inappropriate features include:

- Displacement of mercury column--Mercury unsafe; displacement is gravity dependent.
- Orifice assembly design--Orifice must be immersed in sample to be counted. Requires open vessel.
- Sample aspirated--The counted specimen is aspirated to a separate waste vessel where it is stored until the vessel is full.

Other systems have attempted to eliminate the requirement for mercury with greater or lesser degrees of success. Most of these systems employ photo-electric switches to regulate the volume of sample aspirated or counted. These systems appear to be troubled with a high degree of imprecision due, at least in part, to varying volumes counted. These systems also require the storage of counted specimen in a waste vessel.

The system developed for NASA under this program completely overcomes these objections. Aspiration of specimen is accomplished by withdrawing the plunger of a syringe which is coupled to the orifice assembly with a short length of flexible tubing. The syringe plunger is attached, by means of a fitting, to a ball-screw drive powered by a stepper motor, as further described in Section 3.2.2.

In operation, the syringe plunger is retracted a distance sufficient to fill system dead space and to clean any residual bubbles or foam through the orifice assembly. Following the interval, the counter is enabled for a volume equivalent to 0.43 ml of fluid. Following the aspiration of this volume, the counter is disabled for a short interval until the end of the aspiration stroke. Upon the completion of the aspiration stroke, the syringe drive is reversed, dispensing all fluid back into the sample container. This action serves two useful purposes: (1) No reservoir for waste solution is required. All sample is returned to its original container; and (2) The orifice assembly is washed with sample each sample cycle. This forward and backward movement of fluid prevents buildup of fibrin material that might tend to plug the orifice.

Thus, the objections common to other systems are overcome.

### 3.2.2 Electronic Design

The electronics for the prototype was designed with the following objectives in mind:

1. Small size and weight.
2. Use of Mil Spec parts (some equivalent parts were used in the prototype).
3. Operation from a single dc power source (+24 to +32 Vdc).
4. Simplicity of operation.

The electronics may be broken down into functional sections:

1. Regulators and power control.
2. Preamplifier, amplifier, comparators, and lockout logic.
3. Motor driver.
4. Cell counter.
5. Display.
6. Cell counter control.

The cell counter's operation is illustrated by the flow chart in Figure 3-3. The operator starts the count sequence by pushing the "start" switch. The system aspirates the sample, through the orifice, enables the cell counter

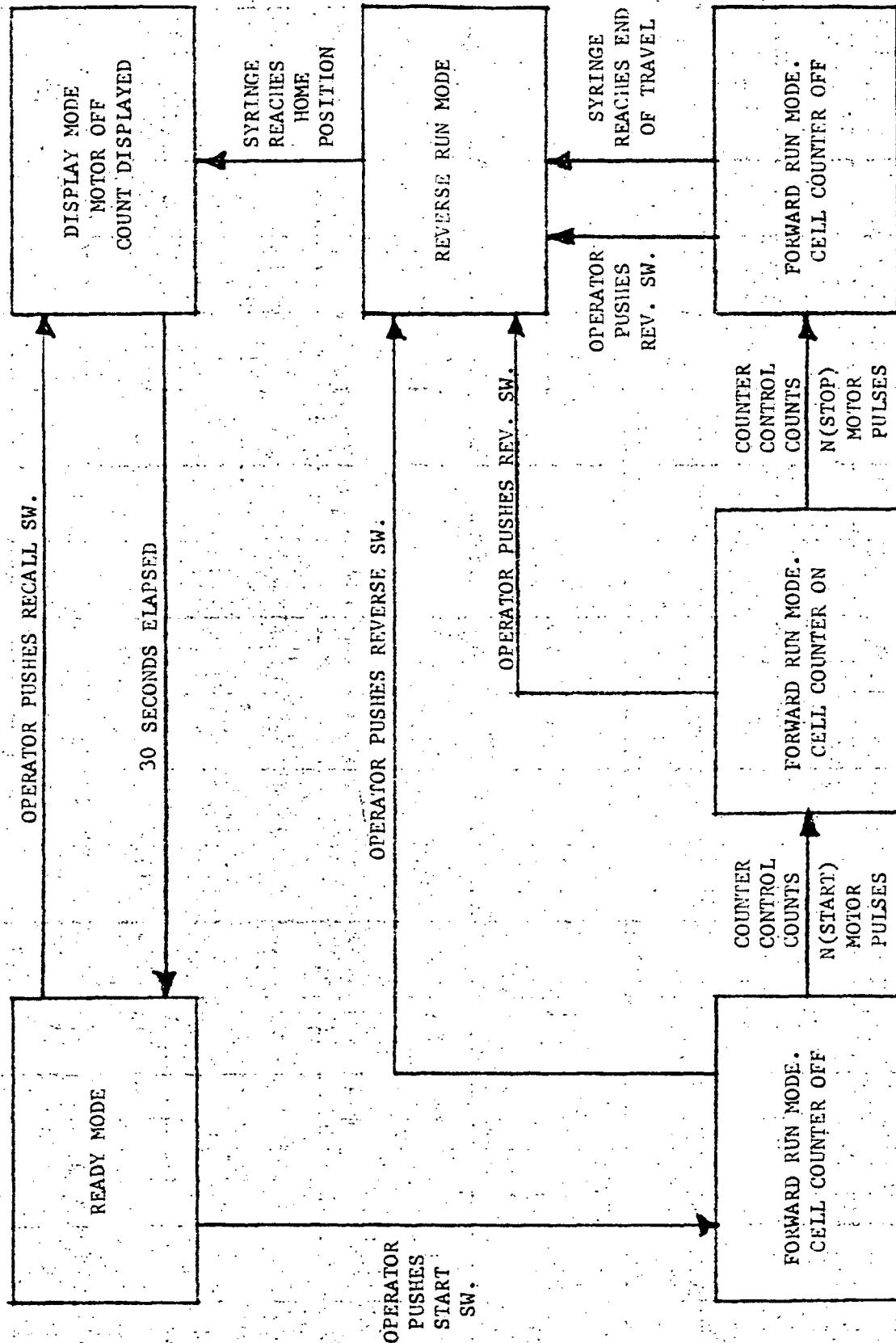


FIGURE 3-3. CELL COUNTER FLOW CHART.

during the proper interval and automatically reverses the mechanism at the end of the travel. When reverse drive brings the syringe back to the home position, the motor is shut off, and the count is displayed for thirty seconds. The information (last count) is retained indefinitely, as long as the power is on, and may be recalled and redisplayed for thirty more seconds by pushing the "display recall" switch.

The operator may terminate the sequence at any point during the cycle by pushing the "reverse" switch. This places the stepper motor in reverse and dispenses any fluid that may have been in the system.

The "ready" light indicates that the system is ready to start a complete cycle.

The "count error" light comes on (after a slight delay) if cell bias current is lost. This light will stay on and can only be reset by turning off the power.

The "orifice fault" light was not connected in the prototype, but could be used in future units. These units might contain a pressure transducer activated by pressure buildup due to a plugged orifice. The purpose would be twofold--to protect the syringe and cell assembly from excess pressure buildup and to alert the operator.

The "reverse" and "count enable" lights are operator aids which indicate what is happening in the cycle and also give some information as to correct system operation.

Figure 3-4 gives a detailed breakdown as to what is happening during each portion of the cycle.

### 3.2.2.1 Regulators and Power Control--Power Conditioning and Distribution

During the "ready" mode, standby +28 V is applied to the standby regulator, which produces standby +5 V. This is applied only to the cell counter circuits in order to preserve any count information which may be present.

The power distribution is controlled by two relays (K1 and K2) on the power control card. During the "ready" mode, K1 and K2 are de-energized. When the

SYSTEM CYCLE STATUS	TIME SEC.	SYR. SPEED IN/SEC.	RDY. LIGHT	REV. LIGHT	DISPLAY	COUNT LIGHT	+5 V STANDBY	+5 V +28 V SW.	+5 V +28 V DIS.	+5 V REG	+5 V REG
Ready		0	On	Off	Off	Off	On	Off	Off	Off	Off
Counter not Enabled	26.5	.0224	Off	Off	Zeros	Off	On	On	On	On	On
Forward Counter Enabled	49	.0224	Off	Off	Count Buildup	On	On	On	On	On	On
Counter not Enabled	0.45	.0224	Off	Off	Final Count	Off	On	On	On	On	On
Reverse	39.2	.0448	Off	On	Final Count	Off	On	On	On	On	On
Display Mode	30	0	On	Off	Final Count	Off	On	Off	On	On	On
Ready	--	0	On	Off	Off	Off	On	Off	Off	Off	Off
Display Recall	30	0	On	Off	Final Count	Off	On	Off	On	On	On

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Figure 3-4. Events Versus Cycle

start switch is pushed, the K1 relay is activated and latches itself into the energized state, in which the K1 contacts apply +28 V to the main +5 V regulator (for all non-standby functions) and the stepper motor. The K1 contacts also route the +5 V from the main 5 V regulator to logic and amplifier circuits not on standby power. When the start switch is pushed, Q5 and C4 (power control card) cause the reset line ( $\bar{R}$ ) to momentarily go to ground. This resets the cell counters, the counter control counter, the counter control flip-flops. The stepper motor driver receives pulses derived from one strobe line of cell counter #2 at a rate of 150 pulses per second and starts to run in the forward direction. As the syringe drive mechanism pulls away from "home" position, the home micro-switch, S1, applies +28 V to K2, which latches itself in the energized position. The K2 contacts now route +5 V from the main 5 V regulator to the display circuits, and route +28 V to the +3.5 V display regulator, which provides +3.5 V for the display LED currents.

When the syringe mechanism reaches the far end of its travel, the reverse micro-switch S2 is actuated and causes the motor control flip-flop to change state. This reverses the motor driver counter, causing the stepper motor to reverse. It also causes the stepper motor driver to receive pulses derived from two strobe lines of cell counter #2, the total of which is 300 pulses per second. This causes the motor to run twice as fast in reverse.

When the motor reaches the "home" position, S1 is again activated, causing the K1 relay drive transistor to be shut off. K1 unlatches and stays de-energized, removing logic and amplifier voltages. K2 is still latched and all display voltages are on.

When the "home" position is reached, a 30 second timer (power control--Q3, C2, CR2, R17, etc.) is started. After approximately thirty seconds, this timer switches Q4 on, which shuts off the K2 relay drive transistor. K2 unlatches and stays de-energized. All voltages except standby are now off.

### 3.2.2.2 Preamplifier, Amplifier, Comparators and Lockout Logic

During the forward running phase, when K1 is latched, the lockout card applies a filtered dc voltage to the cell, which causes about 100 microamps to flow

through the orifice. The voltage generated across the orifice is ac coupled to the preamplifier which has a gain of 100. When a cell passes through the orifice, the resistance increases, causing a small positive voltage pulse to be sent to the preamplifier. The amplified output is ac coupled to the comparator card and amplified another 60 times, then fed to two comparators. The comparators are referenced to high and a low threshold reference voltages, respectively. Either comparator will put out 0 V if the input is below its reference threshold. When the input pulse exceeds the comparator's reference voltage, the comparator output goes to +5 V (logic 1).

The comparator outputs feed logic on the lockout card, which obeys the following logical statement:

$$C = \bar{L}H\bar{E}$$

where      C = logic output to cell counter

              L = input from low threshold comparator

              H = input from high threshold comparator

              E = counter enable signal.

This means that pulses will only be counted when  $E = 1$  ( $E$  is from the counter control circuit).

Only those pulses which are greater than the lower threshold ( $L = 1$ ) and less than the upper threshold ( $H = 0$ ) will be counted.

### 3.2.3     Motor Driver

The stepper motor driver consists of a four bit reversible counter, logic gating and four amplifier-driver Darlington circuits.

The motor clock, as stated before, is derived from cell counter #2 strobe lines (only one strobe line to give 150 pulses/sec in forward or two strobe lines to give 300 pulses/sec in reverse).

The four bit counter consists of two flip-flops "A" and "B". "A" changes state with each clock pulse. "B" changes state with every other clock pulse, but when

the state change occurs depends on the state of  $M_F$ . When  $M_F = 1$ , the sequence in which motor windings is energized is: A, B, C, D, A, B, etc. (motor is in forward).

When  $M_F = 0$ , the sequence is reversed, D, C, B, A, D, C, etc. (motor is in reverse).

### 3.2.4 Cell Counter

Cell counter cards #1 and #2 comprise a decimal counter, capable of counting up to 99,999 cells.

The cell counters are made up of two Mostek 5007P 4-digit counter/decoders. Each of these counters is capable of counting up to 9,999 counts. Two such devices could count up to 99,999,999, but since the display is limited to five decimal digits, the second counter is utilized only for the first decimal digit.

Each Mostek counter has an output of four BCD lines and four strobe lines (one for each of the four decimal digits). The decimal digits are strobed (MSD to LSD) in order. Each time a strobe line comes on, the BCD code is for that decimal digit (see Figure 3-5, which shows a count of 5803).

The count extend line of the first Mostek counter produces a pulse every 10,000 counts. This line (after buffering) is fed to the count input line of the second Mostek counter so that the fifth decimal digit may be counted.

### 3.2.5 Display

The display shows the cell count and consists of five Hewlett-Packard dot-matrix LED displays.

The cell counter feeds the display through buffer stages, and the relationship between the counters and displays is shown in Figure 3-6.

The display is strobed by the cell counters. The most significant digit (MSD) is strobed by cell counter #2 at about 150 times a second, while the four less significant digits are each strobed at about 300 times per second.

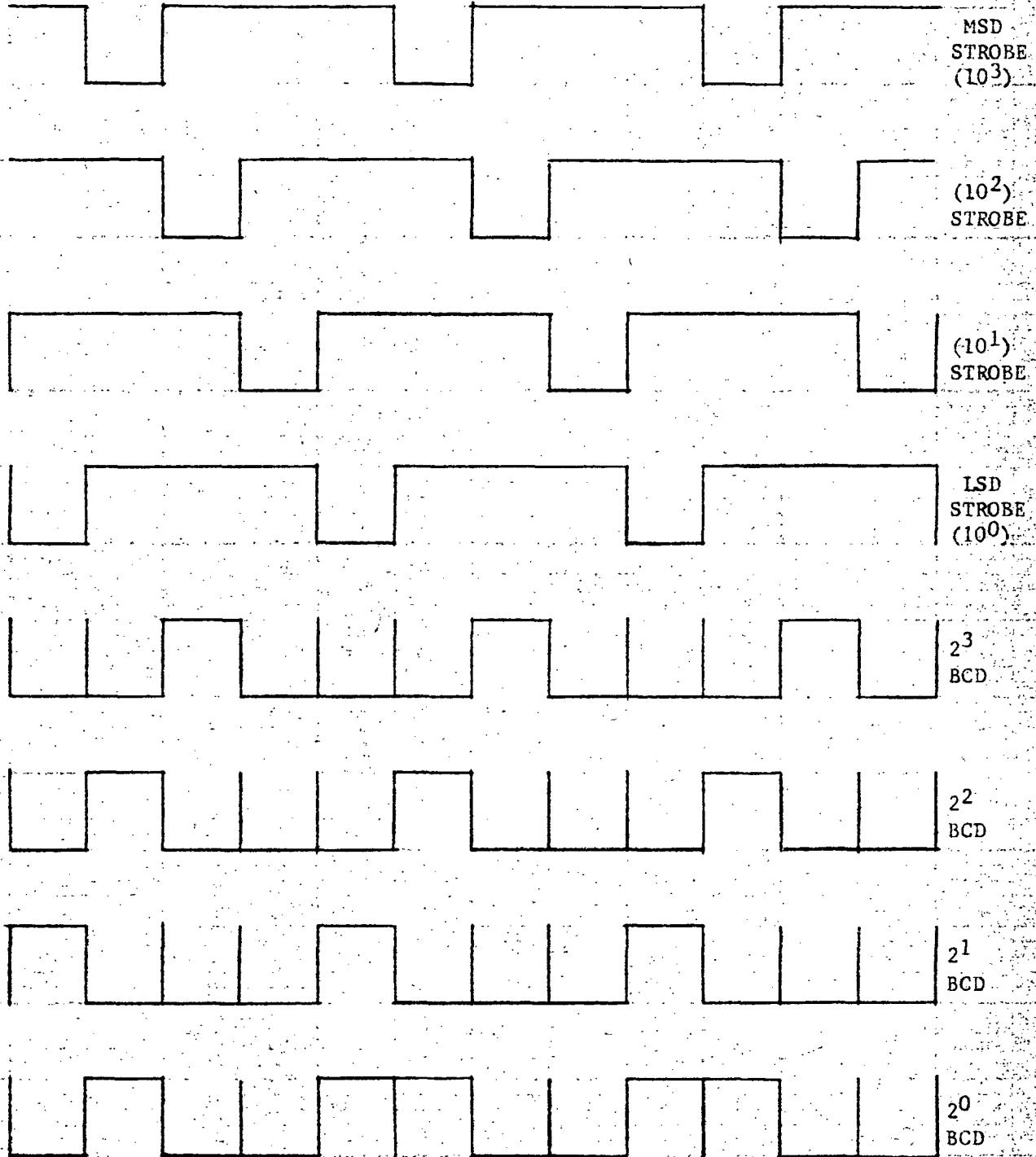


FIGURE 3-5. MOSTEK OUTPUT WAVEFORMS FOR 5803

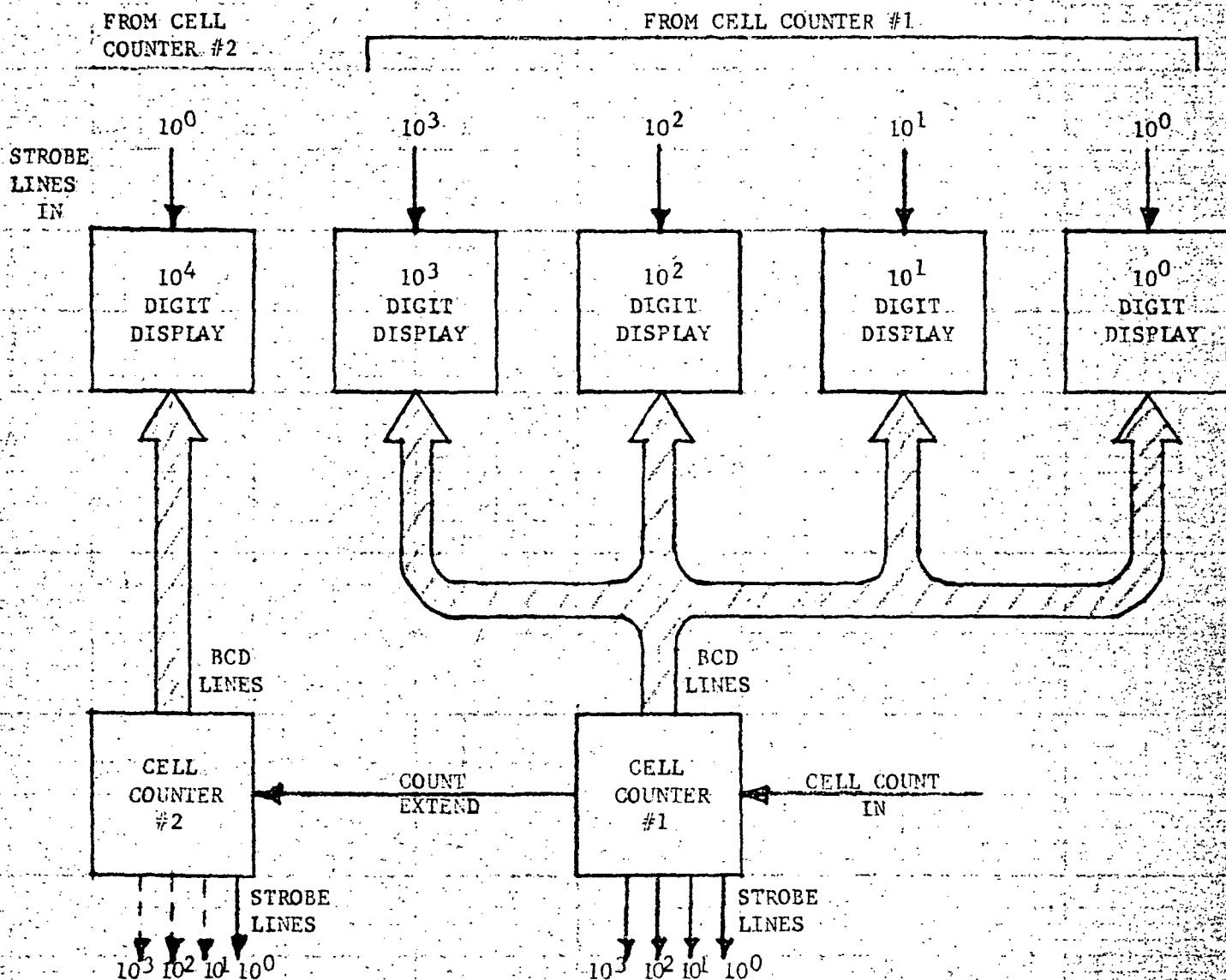


FIGURE 3-6. RELATIONSHIP BETWEEN CELL COUNTERS AND DISPLAYS

### 3.2.6 Cell Counter Control

The cell counter control enables the cell counter during the proper interval during the cycle.

The cell counter control operates by counting motor pulses with a Mostek 5007P. Since the output of the 5007P does not come up all at once, but one decimal digit at a time, a memory system must be used to detect the start and stop count. This is done by the method shown in Figure 3-7.

Both the start and stop counts are programmable by means of jumpers soldered on back of counter control #1.

When the correct number of start motor pulses have been counted, the start flip-flop is set, thus enabling the cell counter. The start flip-flop can only be set with the following counts (since only two gate inputs are used):

$$\text{Start} = N \times D$$

where  $N = 8, 4, 2 \text{ or } 1$

$D = 1000, 100, 10 \text{ or } 1.$

The stop can be set exactly to any count between 0 and 9,999. Assume that the number chosen for a stop count is 9,473. Then, when 9000 is reached, the 1000's flip-flop is set. 9,400 sets the 100's flip-flop. 9,470 sets the 10's flip-flop. 9,473 sets the 1's flip-flop. When the 1's flip-flop sets, it causes the entire ring of start and stop flip-flops to be reset. The entire number of motor pulses counted is  $N(\text{stop}) - N(\text{start})$ . A capacitive coupled circuit senses when the counter enable line goes negative (at the end of the count) and latches in an SCR, thus disabling the Mostek counter. This prevents any additional counting during the reverse part of the cycle, since a second "phantom" count could occur due to the Mostek counter resetting after 9,999 and starting over again.

All clock logic to all flip-flops in the system is derived from one strobe line from cell counter #1 (about 300 cps).

The strobe rate for the counter control counter is about 1400 cps per strobe line.

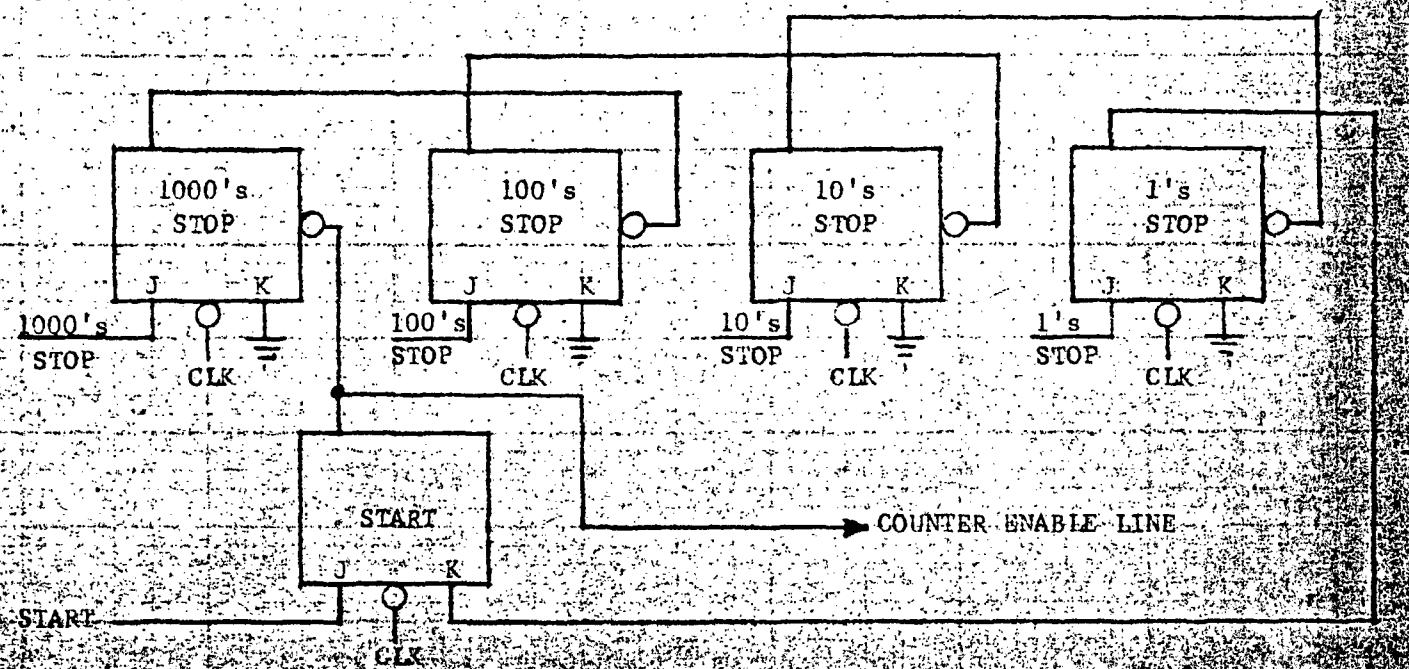
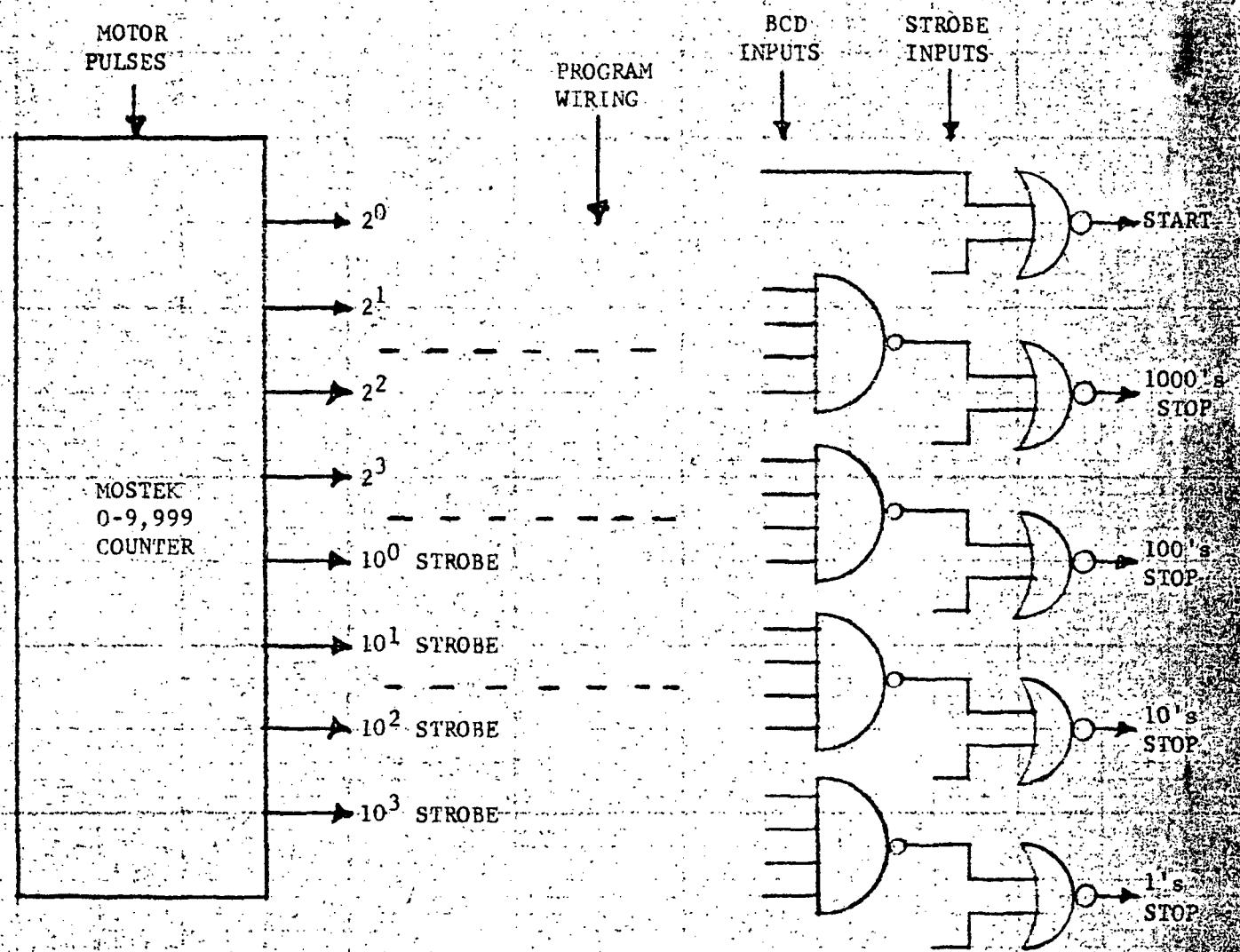


FIGURE 3-7. CELL COUNTER CONTROL

#### 4.0 LABORATORY VERIFICATION STUDIES

Laboratory verification studies were conducted at all stages of the development process and were reported in the Monthly Progress Reports. The results of the testing may be summarized as follows:

- Diluter Subsystem

The diluter subsystem as originally designed was accurate but so difficult to operate that precision was affected. The current design appears to have overcome these problems.

- Counter

The counter shows excellent signal to noise characteristics and correlates very well with counts obtained on a Coulter Counter when the counts are aspirated from a beaker. Further, the precision on such samples is generally superior to that obtained on a Coulter F. Samples aspirated from bags, however, show a broader distribution. It is felt that this is due to inadequate cleaning of the bag material prior to fabrication and that the problem can be overcome with the proper cleaning precautions.

- Bags

As indicated above, the bags appear to contribute a variable error component to the count. In addition, when newly fabricated, they offer a resistance to filling from the diluter assembly. This resistance is not apparent once the bags have been initially expanded with air. It is felt that both of these problems will be resolved with the selection of another bag material, e.g., SLP-4.

#### 5.0 DRAWINGS

Drawings required for fabrication are attached.